

## STUDIES FOR STUDENTS.—II.

THE HISTOBLASTS (IMAGINAL BUDS) OF THE WINGS AND LEGS OF THE GIANT CRANE-FLY (*Holorusia rubiginosa*).

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*Technical note.*—In an old larva of *Holorusia* (or other Tipulid) killed as described in Studies for Students,—I, (PSYCHE, 1901, p. 207,) make a longitudinal cut through the dorsal body wall from head to posterior tip of body and pin out in dissecting dish with ventral aspect down. Remove the fat body and alimentary canal with salivary glands, exposing completely the longitudinal and annular muscle fibers of the body wall. In the thoracic segments carefully pick away these muscle fibers, thus exposing six small white bodies lying, one pair in each thoracic segment, in immediate contact with the skin. These are the histoblasts (imaginal buds) of the wings and legs of the imago.

**Position, and External Characters.**

The *histoblasts*, imaginal buds or imaginal discs, as they are variously termed, of the wings and legs of the imago of *Holorusia* are readily found in the old larvae. They are small, firm, white bud-like bodies\* (fig. 1, A) lying

in the thoracic segments, against the *hypoderm* (cellular skin layer of the body wall) of which they are actual invaginations. There are two pairs in each segment, those of the prothorax being the beginnings of the fore legs and of the pupal dorsal prothoracic respiratory tubes, those of the mesothorax corresponding to the mesothoracic legs and wings, and those of the metathorax to the metathoracic legs and halteres. In the dissection as made, the inner or mesal buds are those of the legs, the outer or lateral ones being those of the respiratory tubes, wings and halteres. The buds of the respiratory tubes and of the balancers are smaller than the others.

By examination under the microscope it may be seen that each bud is connected intimately with the hypoderm by a very short *neck*. The buds may be swayed slightly to one side or the other. If care is taken in dissecting away the viscera and muscles to expose the buds, each will be noted to have a *tracheal tube* running to it.

**Histology and Development.**

For a study of the structure and of the development of the histoblasts it is necessary to make sections (by micro-

\* For the reason that some of these "imaginal" buds are in many insects the beginnings of strictly pupal organs which are not present in the imago, I believe that the name imaginal buds or discs should be discarded. The name, histoblasts, used first, I believe, by Künckel d'Herculais, seems preferable.

tome) of them. For this purpose the skin of the thorax with attached buds should be cut out, either in one piece with all the buds, or in segmental pieces with the buds of one segment, or in six distinct pieces, each bud with a bit of skin by itself. These pieces must be properly hardened, cleared, infiltrated with and imbedded in paraffine, sectioned (cut into thin slices) by means of

ment a longitudinal section (fig. 2, B) i. e., one through skin and bud at right angles to the long axis of the larva, it may be seen that the bud is composed of an invaginated part of the hypoderm layer which has become folded, and in which there has been a special increase and growth of cells. The folding and modification of this part of the hypodermal cell layer is such that part of it,

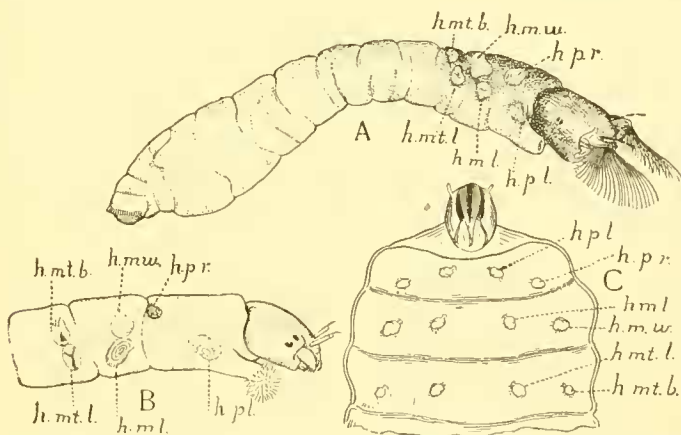


Fig. 1. A, Larva of *Simulium*, showing thoracic histoblasts, as they show through the skin; B, larva of *Chironomus*, similarly showing thoracic histoblasts; C, Head and body wall of thoracic segments of larva of *Holorusia rubiginosa*, showing histoblasts; *h. p. r.* histoblast of prothoracic respiratory tubes of pupa; *h. p. l.* histoblast of prothoracic leg, *h. m. w.* histoblast of wing, *h. m. l.* histoblast mesothoracic leg, *h. mt. b.* histoblast of balancer, *h. mt. l.* histoblast metathoracic leg.

a microtome, and the sections fastened to a glass slide in regular order, stained, cleared, and finally mounted in Canada balsam. For an account of the details of histological technic with special reference to insects, see Comstock and Kellogg's *Elements of Insect Anatomy*, 1899, pp. 111-129.

In any thoracic bud of *Holorusia* which is about midway in its develop-

called the *peripodal membrane*, is very thin (compared with the rest of it) and serves as a partially enveloping membrane, and as the walls of a neck which connects the thickened part of the histoblast with the hypoderm at the point of invagination. It is the thickened part of the invaginated hypoderm (the inner part of the bud) which is to develop into the wing, or leg, or balancer,

or respiratory tube (depending on which bud is being examined). Outside of the bud, i. e., in the body cavity of the larva, and lying in contact with the bud, may be seen the cut end of a tracheal tube, and, adjoining it, a group of cells containing, or rather changing into, a

The histoblasts will be found in different stages of development in larvae of varying ages. For a careful study of the course of development of the histoblasts it will be necessary to obtain a series of larvae from young to fully grown stages, and to section a series of

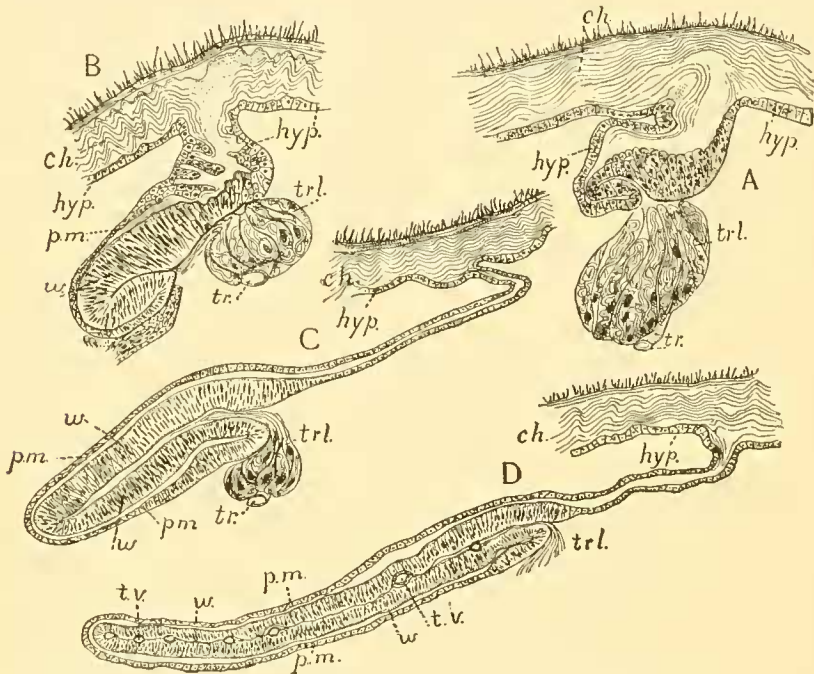


Fig. 2. Histoblast of wing of *Holorusia rubiginosa*, at different stages of development, (in section); A, youngest (of the four stages) B, C, D, successively older stages; *ch.*, chitin layer of dorsal thoracic body wall, *hyp.*, hypoderm, *p. m.*, peripodal membrane, *w.*, actual wing-forming part of the histoblast, *tr.*, trachea, *trl.* tracheoles, *t. v.*, "tracheal vein," (each wing-vein consisting first of a tracheal tube).

mass of fine capillary tubes convoluted and closely massed, called *tracheoles*. Also, there may be noted, perhaps, a few cells called *embryonic cells* which have come from the interior of the body to take part, probably, in the formation of the imaginal wing or leg.

histoblasts taken from these larvae. This development in *Holorusia* is easy to follow, as the larvae may be obtained young and reared in the laboratory, specimens being killed at regular intervals. The size of the buds and the readiness with which they may be dis-

sected out, and sectioned (in any plane desired) makes a detailed study of the development of the wings and legs of *Holorusia* (or any other large Tipulid), a matter readily accomplished by any student who has access to a microtome. The limits of this paper preclude any detailed account of the development. For the orientation of students I figure and briefly describe four stages (fig. 2) in the development of a wing bud, namely, a beginning stage, a middle stage, a later stage, and a stage from a larva nearly ready to pupate. These four stages show all of the parts and the most marked developmental changes of the buds. They should be sufficient to enable a student to interpret correctly the parts and developmental phenomena in a detailed study of the histology and development of the wing-buds of any insect with complete metamorphosis.

In the first stage (fig. 2, A) figured the hypoderm is thickened and invaginated and slightly folded. This folding it will be noted results in a shallow evagination within the cup or cavity formed by the primary invagination. It will be noted that the thickened (bud) layer is perfectly continuous with the normal hypoderm (true skin) which overlies the body just inside of the chitinous cuticle. The neck of the bud is broad and is filled by chitin. The hypoderm layer composing the neck is not thickened, or but slightly. This distinction of thickening is later more emphasized, the thin part being the peripodal membrane, while the thickened part forms the true wing-forming part of the bud.

Just outside of the bud in the body cavity and in immediate proximity to it is a tracheal tube (cut transversely across in the section) and giving off a mass of fine tangled *tracheoles* which tend to push into the concavity at the base of the bud caused by the slight evagination of the basal part of the bud. These tracheoles are formed by a peculiar enlargement and tubule-forming of the cells of the epithelium of the tracheal tube. The nuclei of these cells are large and conspicuous. The tracheoles are simply fine capillary tubules, and lack the spiral thread characteristic of tracheae.

In the next stage (fig. 2, B) figured the folding is more pronounced, resulting in a filling up of the cavity caused by invagination, the neck of the bud is narrower, and the distinction between the peripodal membrane and the true wing forming part of the bud layer more pronounced. The chitin inside the bud (which is of course perfectly continuous with the actual outside of the body) can be traced far down in the bud forming a thin double layer indicating always the true external surface of the developing wing.

The third stage (fig. 2, C) shows the two distinct layers of the wing enclosed by the peripodal membrane, and the long slender "neck" of the bud connecting it with the normal skin hypoderm.

The fourth stage (fig. 2, D) shows the two wing layers in contact and the forming veins (in cross section) along this line of contact. The wing now lies as a well formed wing pad with thick

cellular layers and forming veins within the cup or cavity formed by the peripodal membrane. To assume its normal definitive position in the imago it is only necessary that it be thrust out through the narrow opening, the mouth of the original invagination of the skin hypoderm. This evagination or shifting from apparently inside the body to outside occurs at the time of pupation, the wing thereafter lying folded on the ventral thoracic aspect of the pupa.

### References.

Students undertaking the study of the development of the histoblasts will need to refer to detailed accounts of such development as studied and described by reputable entomologists (and zoologists). For an introduction to, or general abstract of our knowledge of this subject (up to 1897) see "The Imaginal Discs of Insects," by H. S. Pratt, *PSYCHE*, Feb. 1897, vol. 8, no. 250; for a detailed account of the development of the wing discs of a particular insect species see "The Development of the Wings in the Lepidoptera" by W. F. Mercer, *Jour. N. Y. Ent. Soc.*, March 1900, vol. 8, no. 1. For further

references see the bibliography given in these two papers.

### Histoblasts Showing Externally.

The integument of the larva of *Holotrusia* is too opaque to permit the buds to be visible from the outside of the body, and this is the case with most larvae. But in some the thoracic buds may be readily seen from the outside, and the gross details of their development followed by simple examination of the exterior of the larvae. This is true for example of the larvae of *Chironomus* (fig. 1, B) and especially of *Simulium* (fig. 1, A). The position and gross appearance of the thoracic buds in *Simulium* can be seen in young larvae and the growth and the foldings and convolutions of the hypodermal layer followed by examination of successively older larvae. As the larvae of both *Simulium* and *Chironomus* are common all over the country, (*Chironomus* in ponds and still pools of streams, and *Simulium* in clear swift water in dense patches on submerged rocks) some acquaintance at least with imaginal buds can be made without either dissection or sectioning.

## LIFE HISTORIES OF NORTH AMERICAN GEOMETRIDAE.—XXV.

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*Cingilia catenaria* Cram. The mature larva has been described by Harris, Packard, Coquillett and Scudder.

*Eggs.* Laid loosely and falling to the ground. Elliptical, gently flattened on two sides, one end distinctly and sharply trun-